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DATA EVALUATION REPORT

ZIRAM

STUDY TYPE: MULTI GENERATION REPRODUCTION FEEDING - RAT (83-4)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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ZIRAM**Reproduction Study (83-4)**

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CEB2 (7509C)

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CEB2 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat
OPPTS 870.3800 [83-4]

DP BARCODE: D223815SUBMISSION CODE: S501475P.C. CODE: 034805TOX. CHEM. NO.: N/ATEST MATERIAL (PURITY): Ziram (97.8% a.i.)SYNONYMS: Zinc dimethyldithiocarbamate

CITATION: Nemec, M.D. (1996) A dietary two-generation reproduction and developmental neurotoxicity study of ziram in rats, WIL Research Laboratories, Inc., 1407 George Road, Ashland, OH 44805-9281. Laboratory study number WIL-223003, January 30, 1996. MRID 43935801. Unpublished.

SPONSOR: The Ziram Task Force, NPC, Inc., 22636 Glenn Drive, Suite 304, Sterling, VA 22170

EXECUTIVE SUMMARY: Ziram (97.8% a.i.) was administered to male and female Sprague-Dawley CD rats in the diet at concentrations of 0, 72, 207, or 540 ppm for two generations (MRID 43935801). Premating doses for the F₀ males were 5.3, 14.8, and 37.5 mg/kg, respectively and for the F₀ females were 6.1, 16.8, and 42.8 mg/kg, respectively. Premating doses for the F₁ males were 5.6, 16.7, and 42.7 mg/kg, respectively, and for the F₁ females were 6.3, 18.4, and 47.5 mg/kg, respectively. Each generation contained 30 animals/sex/dose which were given test or control diet for at least 10 weeks then mated within the same dose group. F₁ animals were weaned on the same diet as their parents. Sibling matings were avoided and at least 23 litters were produced in each generation. All animals were exposed to test material either in the diet or during lactation until sacrifice. The time course for the study was as follows: weeks 1-10, F₀ premating; weeks 11-18, F₀ breeding, gestation, and lactation; weeks 19-30, F₁ premating; week 39, end of study.

All F₀ and F₁ parental animals survived to scheduled necropsy. Generalized, clinical signs in the adult animals, such as hair loss and sores, were observed in the control and treated animals equally and there was no correlation with dose.

No treatment-related effects were seen in the 72 or 207 ppm groups of either generation as compared with controls. High-dose F₀ males initially had lower body weights (90-93%) than controls at weeks 1, 2 ($p \leq 0.01$), and 3 ($p \leq 0.05$) due to a significantly ($p \leq 0.01$) lower body weight gain (71%) during week 0-1. Throughout the remainder of the study, there were no significant differences in absolute body weights of the treated F₀ male groups as compared

to controls. Food consumption by the high-dose F_0 males was significantly ($p \leq 0.01$) less than controls for the first 4 weeks of the study and at weeks 8-9, 9-10 ($p \leq 0.05$), and 10-11. Body weights of the high-dose F_0 females were significantly ($p \leq 0.01$) less than the controls for the entire premating period (92-94%). However, body weight gains were significantly less than controls only during week 0-1 (44%; $p \leq 0.01$), week 1-2 (76%; $p \leq 0.05$), and week 6-7 (67%; $p \leq 0.01$). High-dose F_0 females ate significantly ($p \leq 0.01$) less than the controls throughout the entire premating period.

High-dose F_1 males had significantly ($p \leq 0.01$) lower body weights (97-90%) as compared to controls throughout the entire premating period and continuing until study termination. Body weight gains in the high-dose males were significantly less than the controls during study weeks 18-19, 20-21 (83%; $p \leq 0.01$), and 21-22 (90%; $p \leq 0.05$) of the premating period. Food consumption was significantly less than the controls for the high-dose F_1 males ($p \leq 0.01$) throughout the entire premating period. Absolute body weights of the high-dose F_1 females were significantly lower than the controls for the entire premating period (89-92%; study weeks 19-23, $p \leq 0.05$; weeks 24-30, $p \leq 0.01$); significantly lower body weight gains (67-87%) occurred only during study weeks 18-19 ($p \leq 0.05$), 23-24, and 24-25 ($p \leq 0.01$). Food consumption by the high-dose F_1 females was also significantly less than the controls throughout premating ($p \leq 0.01$; weeks 21-22 and 28-29, $p \leq 0.05$).

There were no treatment-related gross- or histological abnormalities observed in either generation. Differences in absolute and relative organ weights of the high-dose male and female F_0 and F_1 groups as compared to controls are consistent with reduced body weights of these animals.

Therefore, the systemic toxicity LOAEL is 540 ppm (37.5 mg/kg/day) based on reduced body weights, body weight gains, and decreased food consumption by F_0 and F_1 males and females. The systemic toxicity NOAEL is 207 ppm (14.8 mg/kg/day).

High-dose F_0 animals had significantly ($p \leq 0.01$) lower body weights as compared to controls throughout gestation and until day 14 of lactation; body weight gains were significantly ($p \leq 0.05$) less than controls during the day 10-14 interval of gestation. Some recovery was apparent in the high-dose F_0 females with body weight gains significantly ($p \leq 0.01$) greater than the controls during lactation days 14-21; this resulted in overall body weight gains during lactation significantly greater than the controls. On gestation day 20 and lactation day 21 body weights of the high-dose F_0 animals were 90% and 98% of the control level. High-dose F_0 females also had significantly ($p \leq 0.01$) lower food consumption as compared to controls throughout gestation and during days 4-7 ($p \leq 0.05$) and 7-14 of lactation. The high-dose F_1 females had significantly lower body weights throughout gestation (days 0 and 7, $p \leq 0.05$; day 10, 14, and 20, $p \leq 0.01$) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in the high-dose ($p \leq 0.01$) group as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. On gestation day 20 and lactation day 21 body weights of the high-dose F_1 animals were 89% and 93% of the control level. Food consumption was significantly ($p \leq 0.05$ or $p \leq 0.01$) lower than controls by the high-dose group throughout gestation and lactation.

No dose- or treatment-related effects were noted on the reproductive performance of adults from either generation. F₁ pups from high-dose group dams had consistently lower body weights than controls beginning at day 4 precull with significance (92%; $p \leq 0.01$) reached on day 14. High-dose F₂ pups also had lower body weights than the controls throughout lactation with significance reached on days 1, 4 precull (92-93%; $p \leq 0.05$), 14, and 21 (88-91%; $p \leq 0.01$).

Therefore, the LOAEL for offspring toxicity is 540 ppm (42.8 mg/kg/day) based on reduced pup body weights at birth in F₂ pups and during lactation in both F₁ and F₂ pups. The corresponding NOAEL for offspring toxicity is 207 ppm (16.8 mg/kg/day).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4) in rats.

I. MATERIALS AND METHODS

A. MATERIALS1. Test material: Ziram

Description: white powder
Lot/Batch No.: V528/8331AA
Purity: 97.8%, a.i.
Stability of compound: stable
CAS No.: 137-30-4
Structure:

2. Vehicle and/or positive control

Purina® Certified Rodent Chow® #5002, meal form, was used as vehicle and negative control. No positive control was used in this study.

3. Test animals

Species: rat

Strain: Sprague-Dawley Crl:CD®BR

Age and weight at start of study: approximately 6 weeks; males: 144-218 g, females: 117-174 g

Source: Charles River Breeding Laboratories, Inc., Portage, Michigan

Housing: Parental animals were individually housed in wire mesh cages until mating. Mated females were transferred to plastic maternity cages with nesting material (ground corn cob).

Diet: Purina® Certified Rodent Chow® #5002, meal form, was available *ad libitum*.

Water: Tap water was available *ad libitum* via an automatic watering system.

Environmental conditions:

Temperature: 68 - 79°F

Humidity: 20 - 92%

Air Changes: 10/hour

Photoperiod: 12 hour light/dark

Acclimation period: 12 days

4. Diet preparation and analysis

Fresh diets were prepared weekly and stored refrigerated. All diet formulations were adjusted for the per cent purity of the test article (97.8%). The appropriate amount of test article was added to 5 kg of diet and mixed in a Hobart blender for 5 minutes. This premix was added to a sufficient amount of diet to obtain 17 kg of the appropriate concentration of test diet and blended in a V-twin shell blender for 10 minutes. An intensifier bar was used during the first and last three minutes of this blending time. Samples from each diet were collected weekly and analyzed for concentration. Duplicate samples from the top, middle, and bottom of each dietary mixture were taken prior to initiation of dosing. One set of samples was analyzed for homogeneity while the other was stored refrigerated for 10 days and analyzed for stability.

Results ❖

Homogeneity analysis: Results from the analyses of samples taken from the top, middle, and bottom of each diet formulation showed the preparations to be adequately mixed. Concentrations varied 97.2-107% of target for all formulations.

Stability analysis: Following 10 days at refrigerated storage, the low-, mid-, and high-dose diets were within 76%, 87%, and 96% of their initial measured concentrations. To compensate for the loss in the low- and mid-dose diets, these formulations were fortified by 20% and 15%, respectively. This insured that target concentrations were maintained over the course of the study.

Concentration analysis: Absence of test article was confirmed in the control diet. Concentrations of test article in the low-, mid-, and high-dose diets varied 87-101%, 88-103%, and 96-108% of nominal, respectively. If the measured concentration was >15% from target, fresh diets were prepared.

Results of the dietary analyses indicate that mixing was adequate and that administered concentrations were within 13% of target. Loss of test article in the diet due to storage was compensated for by increasing the initial concentrations of the low- and mid-dose diets.

B. STUDY DESIGN1. In life dates

Start: May 24, 1994; end: March 31, 1995

2. Animal assignment

F₀ animals were randomly assigned to groups by the use of a computerized stratification block design based on body weight. F₁ pups were randomly selected, at least one male and one female from each litter, and weaned onto the same diet as their respective parents. Thirty F₂ pups/sex/group were also randomly selected for developmental neurotoxicity testing; these results are presented in a separate DER. Animal assignment is given in Table 1.

TABLE 1. Animal assignment					
Dose Group	Conc. in Diet ^a (ppm)	No. of Animals per Group			
		F ₀ Generation		F ₁ Generation	
		Male	Female	Male	Female
0 (Control)	0	30	30	30	30
1 (Low)	72	30	30	30	30
2 (Mid)	207	30	30	30	30
3 (High)	540	30	30	30	30

Data taken from pp. 28, MRID 43935801.

^aDiets were administered from the beginning of the study until the animals were sacrificed.

3. Dose selection rationale

Doses were selected on the basis of a dose range-finding study conducted previously by the testing laboratory (WIL-223002). Only data on the analysis of dietary formulations were included with the main study.

C. METHODS1. Mating procedure and schedule

F₀ animals were fed control or treated diets for 10 weeks prior to mating and continuing throughout mating, gestation, and lactation. F₁ animals were weaned onto the same diets as were fed their respective parents. After weaning, the F₁ animals were maintained on treatment for 10 weeks prior to mating. For mating, animals of the same dose group were paired one male to one female. Sibling matings were avoided. Females were examined each morning for evidence of mating which consisted of sperm in a vaginal lavage or a copulatory plug. Day 0 of gestation was designated as the day evidence of mating was seen. Each female was left with its first male for a maximum of 10 days. If no sign of mating was observed, the female was placed with a proven male of the same treatment group for an additional 5 days. When evidence of mating was not detected after the total 15-day period, the female was returned to individual housing.

2. Observation schedule

- a. Parental animals - All animals were observed twice daily for morbidity, mortality, overt signs of toxicity, and dystocia. Body weights of the F₀ and F₁ males were recorded weekly throughout the study and prior to necropsy. Females were weighed weekly until evidence of copulation then on days 0, 7, 10, 14, and 20 of gestation and on days 1, 4, 7, 14, and 21 of lactation. Food consumption for the F₀ and F₁ adults was measured daily until mating. After mating food consumption for males was measured daily until necropsy and for females daily throughout gestation and lactation. Food consumption was calculated and reported as g/animal/day and g/kg/day at weekly intervals. Compound consumption was calculated from the mean food consumption data and the nominal concentration of test article in the diet for each sex/group.

- b. Reproductive performance - The duration of gestation for each female and the following indices were calculated:

Female mating index = (No. females with evidence of mating/Total no. females paired)×100

Male mating index = (No. males with evidence of mating/Total no. males paired)×100

Female fertility index = (No. females pregnant/No. females paired)×100

Male fertility index = (No. males siring a litter/No. males paired)×100

- c. Litter observations - All females were allowed to litter, the pups were examined for gross malformations, and the number of live and dead was pups recorded. Offspring were individually

identified by application of tattoo markings on the digits on lactation day 0. Live pups were counted and examined daily for survival and changes in appearance or behavior. Pups were individually sexed on lactation days 0, 4, and 21. All intact pups dying prior to weaning were necropsied. Each pup was weighed and received a detailed physical examination on lactation days 1, 4, 7, 14, and 21, and at weekly intervals thereafter until euthanasia. On lactation day 4, all litters were standardized to 4 males and 4 females, where possible, and the remaining pups euthanized and discarded. The following indices were calculated:

Live litter size = (No. live pups on lactation day 0/No. litters with viable pups)

Viability index (precull) = (No. live pups at lactation day 1 or 4/No. live pups on day 0)×100

Viability index (postcull) = (No. live pups at lactation day N/No. live pups on day 4 postcull)×100; where N = 7, 14, or 21 [same as lactation index].

3. Postmortem Studies

a. Sacrifice - All animals were euthanized by carbon dioxide inhalation.

b. Necropsy -

- 1) Parental animals - The F₀ adults were euthanized following selection of the F₁ generation. The F₁ adults were euthanized following weaning of the F₂ pups. A complete necropsy and selective histopathological examination was performed on all animals.
- 2) Offspring - Offspring dying from lactation days 0 to 4 were necropsied using a fresh dissection technique. Offspring dying on or after day 4 were subjected to a detailed gross necropsy and tissues saved for histopathological examination as appropriate. All surviving non-selected F₁ pups were euthanized and necropsied on postnatal day 28. All F₂ weanlings not selected for developmental neurotoxicity testing were euthanized and necropsied on postnatal day 22.
- 3) Necropsy observations - F₀ and F₁ parental animals were subjected to a gross necropsy consisting of external and internal examinations. The following tissues (X) were preserved and weighed (XX). All organs and tissues were fixed in 10% neutral buffered formalin. A qualitative evaluation of spermatogenesis was conducted on males rats that failed to sire a litter. The * tissues, as well as the cervix and vas deferens, were examined histologically from the control and high-dose groups. Organ and tissue samples from the F₂ pups were collected and preserved only as deemed necessary by gross findings.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	X	Spinal cord
X	Stomach	X	Lymph nodes	XX	(cervical)
X	Duodenum	X	Spleen	X	Pituitary*
X	Jejunum	X	Thymus		Eyes (optic n.)
X	Ileum				
X	Cecum		UROGENITAL	X	
X	Colon	XX	Kidneys*		GLANDULAR
X	Rectum	X	Urinary bladder	X	Adrenal gland
XX	Liver*	XX	Testes*		Lacrimal gland
	Gall bladder	XX	Epididymides*	X	Mammary gland
X	Pancreas	X	Prostate*		Parathyroids
		X	Seminal vesicle*		Thyroids
	RESPIRATORY	X	Coagulating gland*	X	
X	Trachea	XX	Ovaries*	X	
X	Lung	X	Uterus with	X	OTHER
	Nose		vagina*	X	Bone
	Pharynx				Skeletal muscle
	Larynx				Skin
					All gross lesions and masses*

*These tissues from the control and high dose groups were examined histologically.

D. STATISTICAL ANALYSES

Statistical tests were two-tailed except where noted. Tests were run on a Digital® MicroVAX® 3400 computer. Pup sex ratios, parental mating and fertility indices, numbers of stillborn and dead pups, and pup viability indices were analyzed by a Chi-square test with Yates' correction factor. Parental and pup body weights and parental body weight changes, parental food consumption, mean gestation length, absolute and relative organ weights, and live litter size were analyzed by a one-way analysis of variance (ANOVA) with Dunnett's test. Histopathological findings were analyzed by the Kolmogorov-Smirnov test (one-tailed). Data from nongravid animals was excluded from statistical analyses following the mating period. Significance level was set at 0.05.

II. RESULTS

A. SYSTEMIC TOXICITY

1. Mortality and clinical signs

All F₀ and F₁ parental animals survived to scheduled necropsy. Clinical signs of toxicity in the adult animals consisted of hair loss, sores or scabbing around the ears and eyes, and

malaligned upper incisors. In the F_0 generation, control and treated animals were affected equally and there was no correlation with dose. Hair loss from the limbs was more common in the F_1 male and female high-dose groups than the controls, but this trend was not dose-related.

2. Body weight and food consumption

- a. Premating - Body weight and food consumption data for the F_0 males and females are given in Tables 2 and 3, respectively. High-dose males initially had lower body weights than controls at weeks 1, 2 ($p \leq 0.01$), and 3 ($p \leq 0.05$). This was due to a significantly ($p \leq 0.01$) lower body weight gain (53%) for the high-dose males during week 0-1. Throughout the remainder of the study, there were no significant differences in absolute body weights of the treated male groups as compared to controls. At the end of the premating period, body weights of the high-dose males were 97% of the control value. After week 1, body weight gains of the treated male groups were occasionally greater ($p \leq 0.05$ or 0.01) than the controls. Body weights of

TABLE 2. F ₀ Males: mean body weights and food consumption				
Week of study	Treatment group			
	0 ppm	72 ppm	207 ppm	540 ppm
Body weight (g)				
0	183 ± 13.5	183 ± 14.3	184 ± 13.8	183 ± 14.0
1	234 ± 18.3	234 ± 20.5	231 ± 17.6	210 ± 19.2** (90) ^a
2	275 ± 23.2	279 ± 26.0	278 ± 21.6	252 ± 23.8** (92)
4	341 ± 30.5	352 ± 34.0	353 ± 27.0	323 ± 30.0 (95)
6	389 ± 35.7	403 ± 40.2	404 ± 31.9	372 ± 31.4 (96)
8	429 ± 39.0	447 ± 44.5	448 ± 36.3	417 ± 34.1 (97)
10 (end of premating)	460 ± 40.6	476 ± 45.5	478 ± 35.1	445 ± 34.4 (97)
20 (end of study)	544 ± 40.8	553 ± 57.8	561 ± 52.1	519 ± 45.6 (95)
Weight gain weeks 0-10 ^b	277	293	294	262 (94)
Weight gain weeks 0-20 ^b	361	370	377	336 (93)
Weekly food consumption prior to mating (g/rat/day)				
0-1	24 ± 1.8	24 ± 2.3	23 ± 1.6	17 ± 2.7** (71)
1-2	24 ± 2.2	24 ± 2.6	25 ± 2.0	22 ± 2.3** (92)
3-4	25 ± 1.9	26 ± 2.5	26 ± 1.8	23 ± 2.3** (92)
5-6	25 ± 2.3	26 ± 2.7	26 ± 2.0	24 ± 1.9 (96)
7-8	25 ± 2.2	26 ± 2.8	26 ± 2.3	24 ± 1.9 (96)
9-10	25 ± 2.3	26 ± 2.6	25 ± 2.0	24 ± 2.6* (96)

Data taken from Tables 4 and 10, pp. 92-101 and 114-121, respectively,
MRID 43935801.

^aNumbers in parentheses are percent of control.

^bCalculated by reviewer from week 0 and 10 group means.

Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

TABLE 3. F ₀ Females: mean body weights and food consumption prior to mating				
Week of study	Treatment group			
	0 ppm	72 ppm	207 ppm	540 ppm
Body weight (g)				
0	141 ± 10.4	142 ± 11.6	141 ± 10.4	142 ± 10.4
1	160 ± 10.6	158 ± 11.8	157 ± 13.0	150 ± 9.5** (94) ^a
2	176 ± 11.6	175 ± 13.9	172 ± 14.0	163 ± 10.0** (93)
4	201 ± 13.8	199 ± 16.9	198 ± 14.9	187 ± 10.7** (93)
6	222 ± 16.5	221 ± 20	219 ± 16.0	207 ± 11.9** (93)
8	239 ± 17.9	238 ± 21.9	235 ± 16.6	221 ± 11.4** (92)
10	251 ± 18.8	250 ± 22.4	244 ± 17.6	231 ± 13.0** (92)
Weight gain weeks 0-10 ^b	110	108	103	89 (81)
Food consumption (g/rat/day)				
0-1	17 ± 1.2	17 ± 1.3	16 ± 1.4**	12 ± 1.6** (71)
1-2	16 ± 1.1	16 ± 1.3	16 ± 1.8	15 ± 1.3** (94)
3-4	17 ± 1.8	17 ± 1.3	17 ± 1.7	15 ± 1.2** (88)
5-6	18 ± 1.8	17 ± 1.4	17 ± 1.9	16 ± 1.3** (89)
7-8	17 ± 2.0	18 ± 1.7	17 ± 1.4	16 ± 1.4** (94)
9-10	17 ± 1.5	18 ± 2.7	17 ± 1.5	16 ± 1.5** (94)

Data taken from Tables 4 and 10, pp. 92-101 and 114-121, respectively,

MRID 43935801.

^aNumbers in parentheses are percent of control.

^bCalculated by reviewer from week 0 and 10 group means.

Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

the high-dose F_0 females were significantly ($p \leq 0.01$) less than the controls for the entire premating period. However, body weight gains were significantly less than controls only during week 0-1 ($p \leq 0.01$), week 1-2 ($p \leq 0.05$), and week 6-7 ($p \leq 0.01$). At the end of the premating period, body weights of the high-dose females were 92% of the control value. Body weight gains of the mid-dose females were sporadically greater than or less than ($p \leq 0.05$) the controls.

Food consumption by the high-dose F_0 males was significantly ($p \leq 0.01$) less than controls for the first 4 weeks of the study and at weeks 8-9, 9-10 ($p \leq 0.05$), and 10-11. Week 1 food consumption by high-dose males was 71% of the control amount, but up to 92-96% thereafter. High-dose F_0 females ate significantly ($p \leq 0.01$) less than the controls throughout the entire premating period. Food consumption by the high-dose females for week 1 was 71% of the control amount and ranged from 88-94% thereafter. Mid-dose females had significantly ($p \leq 0.01$) lower food consumption as compared to controls during week 0-1.

Body weight and food consumption data for the F_1 males and females are given in Tables 4 and 5, respectively. High-dose males had significantly ($p \leq 0.01$) lower body weights as compared to controls throughout the entire premating period and continuing until study termination. At the end of the premating period, body weights of the high-dose males were 89% of the control value. Body weight gains in the high-dose males were significantly less than the controls only during study weeks 18-19, 20-21 ($p \leq 0.01$), and 21-22 ($p \leq 0.05$) of the F_1 premating period. Absolute body weights of the high-dose F_1 females were significantly lower than the controls for the entire premating period (weeks 19-23, $p \leq 0.05$; weeks 24-30, $p \leq 0.01$). However, significantly lower body weight gains occurred only during weeks 18-19 ($p \leq 0.05$), 23-24, and 24-25 ($p \leq 0.01$) for the high-dose females as compared to controls. At the end of the premating period, body weights of the high-dose females were 90% of the control value.

Food consumption was significantly less than that of the controls for the high-dose F_1 males ($p \leq 0.01$) throughout the entire premating period and for the mid-dose males ($p \leq 0.05$) for study weeks 18-19 and 19-20. Food consumption by the high-dose F_1 females was also

TABLE 4. F ₁ Males: mean body weights and food consumption				
Week of study	Treatment group			
	0 ppm	72 ppm	207 ppm	540 ppm
Body weight (g)				
19	111 ± 21.8	113 ± 16.2	104 ± 21.5	97 ± 14.7* (87) ^A
21	215 ± 30.0	219 ± 20.7	202 ± 31.3	188 ± 21.7** (87)
23	312 ± 38.3	317 ± 24.0	299 ± 39.8	276 ± 29.9** (88)
25	379 ± 41.2	384 ± 34.2	370 ± 40.2	339 ± 34.5** (89)
27	427 ± 43.5	431 ± 38.6	420 ± 44.0	383 ± 38.2** (90)
30 (end of premating)	477 ± 47.4	478 ± 41.0	471 ± 46.0	426 ± 38.1** (89)
39 (end of study)	550 ± 49.7	549 ± 43.8	548 ± 53.7	494 ± 45.0** (90)
Weight gain weeks 19-39 ^b	439	436	444	397 (90)
Food consumption prior to mating (g/rat/day)				
19-20	19 ± 2.4	19 ± 1.6	18 ± 2.4*	16 ± 1.8** (84)
21-22	23 ± 2.8	24 ± 2.0	23 ± 2.7	21 ± 2.1** (91)
23-24	25 ± 3.2	25 ± 2.2	25 ± 2.7	23 ± 2.3** (92)
25-26	25 ± 2.6	25 ± 2.3	25 ± 2.6	23 ± 2.4** (92)
27-28	26 ± 2.6	26 ± 2.3	25 ± 2.6	23 ± 2.0** (88)
30-31	26 ± 2.6	25 ± 2.0	26 ± 2.6	22 ± 2.0** (85)

Data taken from Tables 32 and 38, pp. 179-188 and 203-210, respectively,
MRID 43935801.

^ANumbers in parentheses are percent of control.

^bCalculated by reviewer from week 19 and 39 group means.

Significantly different from controls, *p ≤ 0.05; **p ≤ 0.01.

TABLE 5. F ₁ Females: mean body weights and food consumption prior to mating				
Week of study	Treatment group			
	0 ppm	72 ppm	207 ppm	540 ppm
Body weight (g)				
19	96 ± 16.7	98 ± 13.4	93 ± 13.4	87 ± 10.4* (91) ^a
21	152 ± 20.8	151 ± 15.7	150 ± 13.3	140 ± 11.4* (92)
23	192 ± 26.9	188 ± 21.5	191 ± 14.5	176 ± 16.5* (92)
25	221 ± 29.1	213 ± 24.3	216 ± 17.3	197 ± 19.5** (89)
27	242 ± 30.9	232 ± 26.2	235 ± 19.4	219 ± 20.9** (90)
30	261 ± 32.3	254 ± 29.5	254 ± 24.1	235 ± 18.7** (90)
Weight gain weeks 19-30 ^b	165	156	161	148 (90)
Food consumption (g/rat/day)				
19-20	16 ± 2.1	16 ± 1.6	16 ± 1.5	15 ± 1.1** (94)
21-22	17 ± 2.3	16 ± 2.4	17 ± 1.7	15 ± 1.2* (88)
23-24	18 ± 2.5	17 ± 2.4	17 ± 1.8	16 ± 1.6** (89)
25-26	18 ± 2.3	17 ± 2.5	17 ± 1.9	16 ± 1.4** (89)
27-28	18 ± 2.1	18 ± 2.3	17 ± 2.0	16 ± 1.6** (89)
30-31	17 ± 2.3	17 ± 2.1	17 ± 2.2	15 ± 1.4** (88)

Data taken from Tables 32 and 38, pp. 179-188 and 203-210, respectively,
MRID 43935801.

^aNumbers in parentheses are percent of control.

^bCalculated by reviewer from weekly group means.

Significantly different from controls, *p ≤ 0.05; **p ≤ 0.01.

significantly less than the controls throughout premating ($p \leq 0.01$; weeks 21-22 and 28-29, $p \leq 0.05$) except at week 24-25 which was not significant.

- b. Gestation and lactation - Body weights, body weight gains, and food consumption during gestation and lactation for the F_0 and F_1 adult females are given in Table 6. High-dose F_0 animals had significantly ($p \leq 0.01$) lower body weights (~90%) as compared to controls throughout gestation and until day 14 of lactation. However, body weight gains were significantly ($p \leq 0.05$) less than controls only during day 10-14 of gestation. Recovery was apparent in the high-dose F_0 females with body weight gains significantly ($p \leq 0.01$) greater than the controls during lactation days 14-21; this resulted in overall body weight gains during lactation significantly ($p \leq 0.01$) greater than the controls. High-dose F_0 females also had significantly ($p \leq 0.01$) lower food consumption as compared to controls throughout gestation and during days 4-7 ($p \leq 0.05$) and 7-14 of lactation. F_0 females in the mid-dose group had significantly lower food consumption than controls on gestation days 0-7 ($p \leq 0.05$) and 14-20 ($p \leq 0.01$) and on lactation days 7-14 ($p \leq 0.05$).

The high-dose F_1 females had significantly lower body weights (89-92%) throughout gestation (days 0 and 7, $p \leq 0.05$; day 10, 14, and 20, $p \leq 0.01$) and lactation ($p \leq 0.01$) as compared to controls. Body weight gains were significantly lower in both the mid- ($p \leq 0.05$) and high-dose ($p \leq 0.01$) groups as compared to controls during days 14-20 of gestation. No significant differences occurred for body weight gains during lactation for any treated group as compared to controls. Food consumption was significantly ($p \leq 0.01$) lower than controls by the high-dose group throughout gestation and by the mid-dose group for the day 14-20 interval. During lactation, the high-dose group ate significantly less food than controls throughout (days 1-4 and 4-7, $p \leq 0.05$; days 7-14 and 14-21, $p \leq 0.01$) and the mid-dose group ate significantly less food during the days 7-14 and 14-21 intervals ($p \leq 0.01$).

TABLE 6. Selected mean body weights, body weight gains, and food consumption values for pregnant and nursing rats fed ziram for two generations

Observation	Treatment group			
	0 ppm	72 ppm	207 ppm	540 ppm
F ₀ Generation				
Mean body weight (g)				
Day 0 of gestation	257 ± 19.5	253 ± 24.9	247 ± 17.0	232 ± 13.0** (90) ^a
Day 20 of gestation	365 ± 32.0	371 ± 32.4	355 ± 25.7	329 ± 23.8** (90)
Day 1 of lactation	284 ± 18.8	283 ± 24.2	277 ± 18.8	259 ± 20.6** (91)
Day 21 of lactation	323 ± 21.3	330 ± 23.2	325 ± 23.4	317 ± 16.1 (98)
Mean body weight gain (g)				
Day 0-20 of gestation	109 ± 25.1	118 ± 21.1	108 ± 18.9	98 ± 15.8 (90)
Day 1-21 of lactation	39 ± 25.1	48 ± 14.2	48 ± 20.1	58 ± 13.4** (149)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation	21 ± 2.1	21 ± 2.0	19 ± 1.4**	17 ± 1.7** (81)
Day 1-21 of lactation	50 ± 6.8	50 ± 4.7	48 ± 4.7	46 ± 3.5* (92)
F ₁ Generation				
Mean body weight (g)				
Day 0 of gestation	258 ± 25.0	264 ± 35.3	253 ± 18.1	237 ± 20.3* (92)
Day 20 of gestation	375 ± 31.0	381 ± 42.9	363 ± 25.7	333 ± 28.8** (89)
Day 1 of lactation	289 ± 25.1	298 ± 35.7	285 ± 22.6	263 ± 28.3** (91)
Day 21 of lactation	324 ± 24.0	326 ± 31.2	319 ± 19.4	302 ± 28.2** (93)
Mean body weight gain (g)				
Day 0-20 of gestation	117 ± 17.5	117 ± 17.3	110 ± 14.7	96 ± 12.7** (82)

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TABLE 6. continued				
Observation	Treatment group			
	0 ppm	72 ppm	207 ppm	540 ppm
Day 1-21 of lactation	35 ± 16.3	28 ± 18.3	34 ± 17.3	39 ± 25.0 (111)
Mean food consumption (g/rat/day)				
Day 0-20 of gestation	20 ± 1.8	20 ± 2.6	18 ± 1.7	16 ± 1.7** (80)
Day 1-21 of lactation	48 ± 3.3	47 ± 4.3	44 ± 4.0**	41 ± 4.7** (85)

Data taken from Tables 6-9, 12, 14, 34-37, 40, and 42, pp. 110-113, 130, 132, 199-202, 223, and 225 respectively, MRID 43935801.

^aNumbers in parentheses are percent of control.

**Significantly different from control, $p \leq 0.01$.

3. Test substance intake

Based on weekly food consumption and nominal Ziram concentrations in the diet, the doses expressed as mg of test substance/kg body weight/day during the premating period for males and females and during gestation and lactation for females are presented in Table 7.

TABLE 7: Test substance intake in rats fed ziram for two generations (mg/kg/day)			
Sex - Study Interval	Concentration in Diet		
	72 ppm	207 ppm	540 ppm
F ₀ Generation			
Males -Premating ^a	5.3 ± 1.3	14.8 ± 3.9	37.5 ± 7.7
Females - Premating ^a	6.1 ± 0.9	16.8 ± 2.7	42.8 ± 4.8
Females - Gestation	5 ± 0.5	13 ± 0.9	33 ± 2.5
Females - Lactation	12 ± 1.2	33 ± 2.6	85 ± 3.3
F ₁ Generation			
Males -Premating ^a	5.6 ± 2.1	16.7 ± 6.0	42.7 ± 14.5
Females - Premating ^a	6.3 ± 1.5	18.4 ± 4.9	47.5 ± 12.5
Females - Gestation	5 ± 0.5	13 ± 0.9	32 ± 2.2
Females - Lactation	11 ± 0.8	30 ± 2.4	79 ± 5.3

Data taken from Tables 16-18 and 44-46, pp. 134-143 and 227-238 MRID 43935801.

^aOverall group means calculated by reviewer from weekly group means.

4. Necropsy results

- a. Organ weights - Selected absolute and relative organ weights for the F₀ males and females are listed in Table 8. High-dose males had significantly ($p \leq 0.01$) increased liver weights relative to body weights. Females in the high-dose group had significantly ($p \leq 0.05$) lower absolute brain weights and increased relative brain weights as compared to controls.

Selected absolute and relative organ weights at necropsy for the F₁ males and females are given in Table 9. High-dose males had significantly ($p \leq 0.01$) lower absolute brain and kidney weights as compared to controls. Males in this group also had significantly increased relative brain ($p \leq 0.05$), epididymides, and testes ($p \leq 0.01$) weights as compared to controls. For females, kidney weights were significantly less than controls in the mid- ($p \leq 0.05$) and high-dose ($p \leq 0.01$) groups. Relative brain weights were significantly greater in the high-dose females ($p \leq 0.01$) and relative ovarian weights were significantly greater in the mid-dose females ($p \leq 0.05$) as compared to controls.

TABLE 8: Selected absolute and relative organ weights from F ₀ rats				
Organ	Concentration in the diet			
	0 ppm	72 ppm	207 ppm	540 ppm
Males				
Brain				
absolute	2.18 ± 0.139	2.17 ± 0.090	2.13 ± 0.089	2.14 ± 0.160
relative	0.402 ± 0.037	0.396 ± 0.040	0.383 ± 0.034	0.414 ± 0.042
Liver				
absolute	19.69 ± 2.090	21.03 ± 3.345	21.39 ± 4.241	20.60 ± 2.694
relative	3.618 ± 0.259	3.792 ± 0.336	3.780 ± 0.438	3.952 ± 0.342**
Females				
Brain				
absolute	1.99 ± 0.126	1.98 ± 0.104	1.96 ± 0.073	1.92 ± 0.051*
relative	0.674 ± 0.057	0.671 ± 0.053	0.691 ± 0.048	0.709 ± 0.051*
Liver				
absolute	11.72 ± 1.267	11.53 ± 1.402	11.46 ± 1.128	11.21 ± 1.070
relative	3.957 ± 0.317	3.894 ± 0.374	4.024 ± 0.290	4.139 ± 0.300

Data taken from Tables 26 and 27, pp. 155-157 and 158-161, respectively, MRID 43935801.

Significantly different from control, *p ≤ 0.05; **p ≤ 0.01.

TABLE 9: Selected absolute and relative organ weights from F ₁ rats				
Organ	Concentration in the diet			
	0 ppm	72 ppm	207 ppm	540 ppm
Males				
Brain				
absolute	2.15 ± 0.082	2.16 ± 0.089	2.13 ± 0.101	2.06 ±
relative	0.387 ± 0.031	0.388 ± 0.030	0.387 ± 0.036	0.111**
				0.411 ±
				0.030*
Kidneys				
absolute	3.94 ± 0.518	3.81 ± 0.410	3.70 ± 0.426	3.52 ±
relative	0.705 ± 0.059	0.683 ± 0.060	0.665 ± 0.046*	0.410**
				0.699 ± 0.065
Epididymes				
absolute	1.41 ± 0.121	1.43 ± 0.103	1.43 ± 0.203	1.41 ± 0.119
relative	0.253 ± 0.025	0.257 ± 0.025	0.258 ± 0.038	0.281 ±
				0.033**
Testes				
absolute	3.73 ± 0.271	3.71 ± 0.293	3.72 ± 0.444	3.69 ± 0.271
relative	0.674 ± 0.075	0.668 ± 0.064	0.673 ± 0.094	0.737 ±
				0.076**
Females				
Brain				
absolute	1.97 ± 0.091	2.01 ± 0.087	1.96 ± 0.094	1.91 ± 0.094
relative	0.641 ± 0.071	0.671 ± 0.060	0.663 ± 0.059	0.699 ±
				0.052**
Kidneys				
absolute	2.36 ± 0.287	2.26 ± 0.238	2.19 ± 0.199*	2.06 ±
relative	0.761 ± 0.061	0.747 ± 0.044	0.742 ± 0.074	0.176**
				0.749 ± 0.043
Ovaries				
absolute	0.141 ± 0.020	0.146 ± 0.019	0.149 ± 0.016	0.132 ± 0.022
relative	0.046 ± 0.008	0.048 ± 0.005	0.050 ± 0.007*	0.048 ± 0.008

Data taken from Tables 49 and 50, pp. 245-247 and 248-251, respectively,
MRID 43935801.

Significantly different from control, *p ≤ 0.05; **p ≤ 0.01.

b. Pathology

1) Gross pathology - In the F₀ males, there was a significant increase in the number of animals in the mid- and high-dose groups with reddened mesenteric lymph nodes: 0/30, 0/30, 6/30 (p ≤ 0.05), and 9/30 (p ≤ 0.01) affected in the control, 72, 207, and 540 ppm groups, respectively.

This effect did not reach statistical significance in the F₁ males, but 0/30, 0/30, 1/30, and 3/30 were affected, respectively. F₁ males also had a dose-related increase in the incidence of mottled lungs with 0/30, 1/30, 2/30, and 5/30 ($p \leq 0.05$) affected in the control, 72, 207, and 540 ppm groups, respectively. No dose- or treatment-related abnormalities were observed in either the F₀ or F₁ females.

2) Microscopic pathology - No treatment-related histological abnormalities were observed for either sex or generation.

B. REPRODUCTIVE TOXICITY

1. Reproductive performance

The reproductive performances of the F₀ and F₁ animals are summarized in Tables 10 and 11, respectively. No dose- or treatment-related effects were noted in either generation. However, F₁ males in the control group had a low fertility index due to only 23 of 30 animals siring litters.

TABLE 10. Reproductive performance in F ₀ generation rats fed ziram for two generations				
Observation	Dietary concentration			
	0 ppm	72 ppm	207 ppm	540 ppm
Mean nights to positive mating	3.6 ± 3.22	2.9 ± 2.29	2.7 ± 1.56	2.7 ± 1.33
Males				
Number paired	30	30	30	30
Number siring	26	26	26	28
Females				
Number paired	30	30	30	30
Number pregnant	27	27	28	28
Number with sperm not detected, littered	1	0	1	0
Number delivering	27	27	28	28
Indices (%)				
Male fertility index	86.7	86.7	90.0	93.3
Female fertility index	90.0	90.0	93.3	93.3
Male mating index	90.0	93.3	96.7	93.3
Female mating index	96.7	96.7	96.7	93.3
Mean gestation length (days)	21.6 ± 0.50	21.6 ± 0.49	21.7 ± 0.48	21.8 ± 0.39

Data taken from Tables 2 and 19, pp. 85-87 and 144, respectively, MRID 43935801.

TABLE 11. Reproductive performance in F ₁ generation rats fed ziram for two generations				
Observation	Dietary concentration			
	0 ppm	72 ppm	207 ppm	540 ppm
Mean nights to positive mating	3.4 ± 2.96	3.1 ± 2.67	2.6 ± 1.17	2.8 ± 2.01
Males				
Number paired	30	30	30	30
Number siring	23	24	26	28
Females				
Number paired	30	30	30	30
Number pregnant	23	25	26	28
Number with sperm not detected, littered	1	2	0	1
Number delivering	23	25	26	28
Indices (%)				
Male fertility index	76.7	80.0	86.7	93.3
Female fertility index	76.7	83.3	86.7	93.3
Male mating index	90.0	90.0	93.3	93.3
Female mating index	93.3	93.3	93.3	96.7
Mean gestation length (days)	21.8 ± 0.43	21.7 ± 0.45	21.8 ± 0.43	21.7 ± 0.48

Data taken from Tables 30 and 47, pp. 171-173 and 239, respectively, MRID 43935801.

2. Viability and clinical signs

Viability data for the F_0 generation offspring (F_1 pups) are given in Table 12 and for the F_1 generation offspring (F_2 pups) in Table 13. The percentage of F_1 male pups was significantly lower in the high-dose group than that of the control. Viability of pups in the low-dose F_0 group was significantly less than the control on lactation day 4 before culling. Pup deaths in this group did not correlate with any clinical or gross necropsy observation.

No differences were observed between treated and control groups for the F_1 generation offspring. The reduced viability index in all groups at lactation day 21 reflects the removal of 10 pups/sex/group on lactation day 11 for neuropathological evaluation and/or brain weight measurement (see corresponding DER).

TABLE 12: Viability of F ₀ generation offspring during lactation				
Observation/ study time	0 ppm	72 ppm	207 ppm	540 ppm
Number of litters	27	27	28	28
Total number of pups	369	364	360	337
Number of pups born alive	367	362	359	336
Number of pups still born	2	2	1	1
Sex ratio (% male) ^a	54.2	51.4	48.2	45.2*
Mean number live pups/litter (day 0)	13.6	13.4	12.8	12.0*
Day 1 ^a	13.4	13.1	12.6	12.0
Day 4 (precull) ^a	13.3	12.6	12.5	11.9
Day 4 (postcull) ^a	7.8	7.9	7.9	7.9
Day 14 ^a	7.8	7.7	7.9	7.9
Day 21 ^a	7.8	7.7	7.8	7.9
Number of litters weaned	27	27	28	28
Survival indices (%)				
Live birth index ^a	99.5	99.5	99.7	99.7
Viability index (precull; d 0-4)	97.8	94.2*	97.8	98.8
Viability index (postcull; d 4-21)	100	98.6	98.6	100

Data taken from Table 20, pp. 145 and 146, MRID 43935801.

*Significantly different from control, $p \leq 0.05$.

^aCalculated by reviewer from group summaries.

TABLE 13: Viability of F ₁ generation offspring during lactation				
Observation/study time	0 ppm	72 ppm	207 ppm	540 ppm
Number of litters	23	25	26	28
Total number of pups	285	310	322	336
Number of pups born alive	281	306	320	333
Number of pups still born	4	4	2	3
Sex ratio (% male) ^a	47.0	44.1	53.4	45.3
Mean number live pups/litter (day 0)	12.2	12.2	12.3	11.9
Day 1 ^a	12.1	12.2	12.2	11.8
Day 4 (precull) ^a	12.1	12.1	12.2	11.7
Day 4 (postcull) ^a	7.9	8.0	8.0	8.0
Day 14 ^a	7.0	7.2	7.2	7.2
Day 21 ^a	7.0	7.2	7.2	7.2
Number of litters weaned	23	25	26	28
Survival indices (%)				
Live birth index ^a	98.6	98.7	99.4	99.1
Viability index (precull; d 0-4)	98.9	98.7	98.8	98.5
Viability index (postcull; d 4-21)	89.0	89.9	90.4	90.6

Data taken from Table 52, pp. 259 and 260, MRID 43935801.

^aCalculated by reviewer from group summaries.

3. Body weight

Selected body weights of the F₁ and F₂ pups during lactation are given in Table 14. F₁ pups from high-dose group dams had consistently lower body weights than controls beginning at day 4 precull with significance ($p \leq 0.01$) reached on day 14. High-dose F₂ pups also had lower body weights than the controls starting at birth and continuing throughout lactation with significance reached on days 1, 4 precull ($p \leq 0.05$), 14, and 21 ($p \leq 0.01$).

TABLE 14: Selected group mean body weights of offspring during lactation (g)				
Day of lactation	0 ppm	72 ppm	207 ppm	540 ppm
F ₁ generation				
Day 1	6.4 ± 0.75	6.5 ± 0.50	6.4 ± 0.52	6.4 ± 0.56
Day 4 (precull)	9.1 ± 1.41	9.3 ± 0.94	8.8 ± 0.93	8.8 ± 1.16
Day 4 (postcull)	9.1 ± 1.37	9.3 ± 0.95	8.8 ± 0.96	8.8 ± 1.15
Day 14	31.4 ± 3.73	32.2 ± 2.82	29.7 ± 3.04	28.8 ± 2.82**
Day 21	43.1 ± 5.03	45.8 ± 3.80	41.5 ± 4.83	40.4 ± 3.53
F ₂ generation				
Day 1	6.7 ± 0.61	6.6 ± 0.52	6.6 ± 0.74	6.2 ± 0.48*
Day 4 (precull)	9.6 ± 1.28	9.8 ± 0.90	9.5 ± 1.20	8.8 ± 0.91*
Day 4 (postcull)	9.5 ± 1.32	9.8 ± 0.93	9.5 ± 1.23	8.9 ± 0.94
Day 14	32.9 ± 2.87	33.1 ± 2.60	31.6 ± 3.28	30.0 ± 3.03**
Day 21	46.8 ± 3.94	47.4 ± 3.60	45.1 ± 4.44	41.0 ± 4.68**

Data taken from Tables 23 and 55, pp. 149 and 263, respectively, MRID 43935801.

Significantly different from control, *p ≤ 0.05; **p ≤ 0.01.

4. Testicular function and sperm assessment

Qualitative assessment of spermatogenesis of the F₀ and F₁ males not siring a litter did not show any dose- or treatment-related effect as compared with controls.

III. DISCUSSION

Male and female Sprague-Dawley rats were fed up to 540 ppm ziram in the diet for two generations. At least 23 litters were produced in each generation.

A. SYSTEMIC TOXICITY

No overt clinical signs of toxicity were observed in the adult animals of either sex or generation. However, high-dose males and females had significantly lower body weights as compared to controls. In the F₀ generation, reduced body weights directly correlated with lower food consumption especially during the initial few weeks of the study. This reduction in food consumption resulted in significantly decreased body weight gains for the first two weeks. Following week 1, body weight gains of the treated male groups were occasionally greater than the controls with recovery of absolute body weights to within 5% of

the control level. Body weights of the F_0 females were also less than the control values during the premating period, but final body weights were within 8% of the control level. Although the initial body weights of the high-dose F_1 males and females were significantly less than the controls, results for the premating period were similar to the F_0 generation. Reduced food consumption by the high-dose F_1 animals caused a reduction in body weight gains resulting in lower absolute body weights with the effect more pronounced during the first few weeks. Mid-dose F_0 females and F_1 males also had significantly reduced food consumption during the first week of feeding. Therefore, the reduced food consumption is most likely due to lack of palatability of ziram to rats.

Changes in absolute and relative organ weights of the high-dose groups did not correlate with gross or histological findings and are probably due to the lower body weights of these animals instead of a direct effect by the test article. At necropsy a dose-related increase in the incidence of reddened mesenteric lymph nodes was observed in males of both generations. However, histological examination was not performed on these organs. An expanded microscopic analysis of the affected lymph nodes might aid in determining whether the effect was directly compound related or due to some other secondary response. This effect was not observed in a subchronic feeding study in rats (MRID 42450301) at a maximum dose tested of 1000 ppm.

Therefore, the systemic toxicity LOAEL is 540 ppm based on reduced body weights, body weight gains, and decreased food consumption by F_0 and F_1 males and females. The systemic toxicity NOAEL is 207 ppm.

Although decreased food consumption also reached statistical significance in the mid-dose group, the effect only occurred during the first week of feeding. A corresponding effect on body weight was not observed at this dose. This supports the lack of palatability of ziram to rats and is not considered as a basis for establishing the LOEL.

B. REPRODUCTIVE TOXICITY

A similar profile of decreased food consumption, reduced body weight gains, and lower absolute body weights was observed in pregnant and nursing rats of both generation. There were no dose- or treatment-related effects on the reproductive performance of either generation. The low male and female fertility indices for the control F_1 parental animals is still within the historical control incidence for the testing laboratory. Excess F_1 pup deaths in the low-dose group are unexplained but do fall within the historical control range. Since pup deaths were not dose related or repeated in the second generation, this effect is probably not treatment related. The low sex ratio among the high-dose F_1 pups was significantly different from the control value, but, the percent male pups for both the control and high-dose groups are within the historical control range (See Appendix 1).

Pup body weights in the high-dose groups were consistently lower than the controls throughout lactation in both generations. Statistical significance was only reached at day 14 for the F₁ pups but the F₂ pup body weights were significantly less than the control at each interval except day 4 postcull.

Therefore, the LOAEL for offspring toxicity is 540 ppm (42.8 mg/kg/day) based on reduced pup body weights at birth in F₂ pups and during lactation in both F₁ and F₂ pups. The corresponding NOAEL for offspring toxicity is 207 ppm (16.8 mg/kg/day).

C. STUDY DEFICIENCIES

Data for F₁ animals were reported as week of study and not normalized for age. However all litters of a treatment group were born within 15 days of each other so at the time of breeding of the F₁ animals sexual maturity would have been reached.

An expanded histopathology of the mesenteric lymph nodes might cause the LOAEL/NOAEL values to change for systemic toxicity. However, the most pronounced effects are still on food consumption and body weight changes.

D. CORE CLASSIFICATION

This study is classified as acceptable and satisfies the guideline requirements for a reproduction study (83-4) in rats.

ZIRAM

Reproduction Study (83-4)

SignOff Date:	8/2/00
DP Barcode:	D172447
HED DOC Number:	014277
Toxicology Branch:	RAB2